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COMPARISON OF hCRF and oCRF EFFECTS ON CARDIOVASCULAR RESPONSES AFTER CENTRAL, PERIPHERAL AND IN VITRO APPLICATIONS. R.M. Richter, M.J. Mulvany[1]. Institute of Molecular Pharmacology, 10315 Berlin, Germany and [1]Danish Biomembrane Research Centre and Department of Pharmacology, Aarhus University, 8000 Aarhus C., Denmark.

The 41-amino acid neuropeptide CRF, originally isolated and sequenced from ovine hypothalamus by Vale et al., is widely distributed in both the central nervous system and peripheral tissues, and modified analogues were found in many species. CRF causes secretion of corticotropin from pituitary gland, evokes prominent stress responses as well as marked cardiovascular effects. Centrally administered CRF produces increased arterial blood pressure and heart rate, while peripherally administered CRF evokes hypotension and tachycardia.

Interest in investigating the biological activity of analogs of CRF continues to increase with the search for the regions of the CRF molecule which are necessary for full biological function.

Therefore, three assays have been used to show that the neuropeptides human corticotropin-releasing factor (hCRF) and the ovine analogue oCRF produced substantial dose-dependent cardiovascular responses. The assays included intracerebroventricular (i.c.v.) and intravenous (i.v.) administration in conscious rats, and also in vitro experiments with resistance arteries.

Central administration of the peptides (0.1-10 µg, i.c.v.) caused an increase in blood pressure and heart rate, while peripheral administration (0.75-750 µg/kg, i.v.) produced a decrease in blood pressure and tachycardia. Isometric ring preparations of mesenteric resistance arteries (diameter 200 µm) relaxed in response to both peptides (1-100 nM). In all cases, the effects were more pronounced for hCRF compared to oCRF. Furthermore, all effects were inhibited by the CRF analogue α-helical CRF(9-41), the effect of the analogue being most potent against oCRF. The results of all three assays indicate that the difference in structure between hCRF and oCRF produces differences in biological activity.

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EFFECT OF THREE ECTOPEPTIDASES ON CRF: METABOLISM AND FUNCTIONAL ASPECTS. J.C. Ritchie, C.B. Nemeroff. Dept. of Pharmacology, Duke University, Durham, NC and Dept. of Psychiatry, Emory University, Atlanta, GA.

Corticotropin-Releasing Factor (CRF) is the primary chemical messenger used by the central nervous system to control the pituitary-adrenal axis. Additionally, there is now considerable evidence that CRF is also synthesized within neurons, at several extrahypothalamic sites, and in the periphery.

Little is known about the termination of action of this 41 amino acid peptide at its receptor. Endocytosis of the CRF-receptor complex is known to occur in the rat anterior pituitary but the actions of the ectopeptidases on this important peptide have not previously been characterized.

This study explored the action of angiotensin converting enzyme (ACE, E.C. 3.4.15.1), aminopeptidase N (APN, E.C.3.4.11.2), and endopeptidase 24.11 (NEP, E.C.3.4.24.11) on CRF. We also evaluated the effects of specific inhibition of these enzymes on CRF function in rat anterior pituitary cultures. Additionally, the role of these ectopeptidases in the regional brain metabolism of CRF was evaluated using specific inhibitors. K_m's were determined for each enzyme, as well as, the characterization of the CRF metabolite profile for each. Though ACE was capable of acting on CRF, its K_m (216µM) cast doubt on the physiological significance of this enzyme. In rat anterior pituitary cultures, inhibition of both NEP and APN were effective in potentiating CRF stimulated ACTH release whereas ACE inhibition was effective only with prolonged incubation. Inhibition of these enzymes in membrane preparations, derived from rat pituitary, hypothalamus, and cerebral cortex indicated that both NEP and APN were involved in CRF hydrolysis in the pituitary and hypothalamus. In cerebral cortex however, inhibition of the three peptidases was without effect on CRF digestion. These data taken together, are consistent with the hypothesis that the ectopeptidases play a major role in CRF metabolism and function.